

# ARCHITECT

## SYSTEM



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## CA 15-3

**REF** 2K44

016-513 4/09

Read Highlighted Changes  
Revised November, 2009

# CA 15-3

**Customer Service: Contact your local representative or find country specific contact information on [www.abbottdiagnostics.com](http://www.abbottdiagnostics.com)**

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

### Key to symbols used

<b>REF</b>	List Number	<b>LOT</b>	Lot Number
<b>IVD</b>	<i>In Vitro</i> Diagnostic Medical Device		Expiration Date
	Store at 2-8°C	<b>CAL A</b>	Calibrator (A-F)
	Consult instructions for use	<b>CONTROL L</b>	Control Low, High (L, H)
<b>SN</b>	Serial Number	<b>ASSAY CD-ROM</b>	Assay CD-ROM
<b>EC REP</b>	Authorized Representative	<b>REACTION VESSELS</b>	Reaction Vessels
	Manufacturer	<b>SAMPLE CUPS</b>	Sample Cups
		<b>SEPTUM</b>	Septum
		<b>REPLACEMENT CAPS</b>	Replacement Caps
		<b>REAGENT LOT</b>	Reagent Lot

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

**WARNING:** CA 15-3 assay values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the CA 15-3 assay used. If, in the course of monitoring a patient, the assay method used for determining serial CA 15-3 levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

## NAME

ARCHITECT CA 15-3

## INTENDED USE

The ARCHITECT CA 15-3 assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of DF3 defined antigen in human serum and plasma on the ARCHITECT *i* System. The ARCHITECT CA 15-3 assay is to be used as an aid in the management of Stage II and III breast cancer patients. Serial testing for patient CA 15-3 assay values should be used in conjunction with other clinical methods for monitoring breast cancer.

## SUMMARY AND EXPLANATION OF TEST

The ARCHITECT CA 15-3 assay values are defined by using the 115D8 and DF3 monoclonal antibodies.<sup>1,2</sup> Monoclonal antibody 115D8, raised against human milk-fat globule membranes, and monoclonal antibody DF3, raised against a membrane enriched fraction of metastatic human breast carcinoma, react with epitopes expressed by a family of high molecular weight glycoproteins designated as polymorphic epithelial mucins (PEMs).<sup>3-6</sup>

Research studies have indicated that CA 15-3 assay values are frequently elevated in patients with breast cancer.<sup>7-17</sup> These studies have suggested that the CA 15-3 assay may be of clinical value for monitoring the response of patients undergoing therapy because increasing and decreasing values correlated with disease progression and regression, respectively.<sup>1,7,10,15-18</sup> Additional published studies have suggested that increasing CA 15-3 assay values in patients at risk for breast cancer recurrence after primary therapy may be indicative of recurrent disease before it can be detected clinically.<sup>10,15,16,19</sup>

Elevations of CA 15-3 assay values have been reported in individuals with nonmalignant conditions such as cirrhosis, hepatitis, autoimmune disorders, and benign diseases of the ovary and breast.<sup>7,8</sup> Non-mammary malignancies in which elevated CA 15-3 assay values have been reported include lung, colon, pancreatic, primary liver, ovarian, cervical, and endometrial.<sup>7,20</sup> CA 15-3 assay values are not elevated in most normal individuals.<sup>7</sup>

The CA 15-3 assay is not recommended as a screening procedure to detect cancer in the general population; however, use of the CA 15-3 assay as an aid in the management of breast cancer patients has been reported.<sup>7-19</sup>

## BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT CA 15-3 assay is a two-step immunoassay to determine the presence of DF3 defined antigens in human serum and plasma, using CMIA technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample, wash buffer and 115D8 coated paramagnetic microparticles are combined. DF3 defined antigen present in the sample binds to the 115D8 coated microparticles. After washing, DF3 acridinium-labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of DF3 defined antigen in the sample and the RLUs detected by the ARCHITECT *i*\* optical system.

**This assay is unique in that the calibrators are supplied prediluted. The ARCHITECT System dilutes all controls and specimens by the same final dilution factor as the prediluted calibrators during the course of the assay.**

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

\* *i* = immunoassay

## REAGENTS

### Reagent Kit, 100 Tests/500 Tests

**NOTE:** Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

### ARCHITECT CA 15-3 Reagent Kit 2K44

- **MICROPARTICLES** 1 or 4 Bottle(s) (6.6 mL for the 100 test bottle/27.0 mL for the 500 test bottle) 115D8 (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine) stabilizer. Preservative: antimicrobial agent.
- **CONJUGATE** 1 or 4 Bottle(s) (5.9 mL for the 100 test bottle/26.3 mL for the 500 test bottle) DF3 (mouse, monoclonal) acridinium-labeled conjugate in phosphate buffer with protein (bovine) stabilizer. Minimum concentration: 0.05 µg/mL. Preservative: antimicrobial agent.

### Other Reagents

#### ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

#### ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

#### ARCHITECT *i* Wash Buffer

**NOTE:** Bottle and volume vary based on order.

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservative: antimicrobial agent.

### Assay Diluent

#### ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50)

- **MULTI-ASSAY MANUAL DILUENT** 1 Bottle (100 mL) ARCHITECT *i* Multi-Assay Manual Diluent containing phosphate buffered saline solution. Preservative: antimicrobial agent.

## WARNINGS AND PRECAUTIONS

- **IVD**
- **For In Vitro Diagnostic Use**
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

### Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens<sup>21</sup>, Biosafety Level 2<sup>22</sup> or other appropriate biosafety practices<sup>23,24</sup> should be used for materials that contain or are suspected of containing infectious agents.
- The microparticles and conjugate contain a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (3:1), which is a component of ProClin 300, and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.




- |     |   |
|-----|---|
| R43 | May cause sensitization by skin contact.  |
| S24 | Avoid contact with skin.  |
| S35 | This material and its container must be disposed of in a safe way.              |
| S37 | Wear suitable gloves.   |
| S46 | If swallowed, seek medical advice immediately and show this container or label. |

- This product contains sodium azide. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.
- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

### Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the ARCHITECT CA 15-3 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- **To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.**
- Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

### Storage Instructions

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- **2°C - 8°C** The ARCHITECT CA 15-3 Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
  - When stored and handled as directed, reagents are stable until the expiration date.
  - The ARCHITECT CA 15-3 Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
  - Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If any reagent bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded. For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.**

### Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

### INSTRUMENT PROCEDURE

- The ARCHITECT CA 15-3 assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* Assay CD-ROM Addition A prior to performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

### SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Only human serum (including serum collected in serum separator tubes [SST]) or plasma (collected in tripotassium EDTA, sodium heparin, or lithium heparin collection tubes) may be used in the ARCHITECT CA 15-3 assay. Other anticoagulants have not been validated for use with the ARCHITECT CA 15-3 assay. Follow the manufacturer's processing instructions for collection tubes.

- When serial specimens are being evaluated, the same type of specimen should be used throughout the study.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen type is used in the ARCHITECT CA 15-3 assay.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Do not use grossly hemolyzed specimens.
- Do not use heat-inactivated specimens.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum and plasma specimens should be free of fibrin, red blood cell or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- If testing will be delayed more than 24 hours, remove serum or plasma from the clot, serum separator or red blood cells. Specimens may be stored for up to 7 days at 2-8°C prior to being tested. If testing will be delayed more than 7 days, serum or plasma should be stored frozen at -20°C or colder.
- Multiple freeze/thaw cycles of specimens should be avoided. Specimens must be mixed THOROUGHLY after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Sample from the middle of the tube to avoid any particulate on the top or bottom of the sample.
- Performance has not been established using body fluids other than human serum and plasma.
- Specimens with obvious microbial contamination should not be used.
- Prior to shipment, it is recommended that specimens be removed from the clot, serum separator or red blood cells. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances. Specimens may be shipped on wet or dry ice.

### PROCEDURE

#### Materials Provided

- 2K44 ARCHITECT CA 15-3 Reagent Kit

#### Materials Required but not Provided

- ARCHITECT *i* System
- 3K50 ARCHITECT *i* **ASSAY CD-ROM** -US- Addition A
- 3K52 ARCHITECT *i* **ASSAY CD-ROM** -WW (excluding US)- Addition A
- 2K44-01 ARCHITECT CA 15-3 Calibrators
- 2K44-10 ARCHITECT CA 15-3 Controls
- 7D82-50 ARCHITECT *i* **MULTI-ASSAY MANUAL DILUENT**
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.
- For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

### Assay Procedure

- Before loading the ARCHITECT CA 15-3 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment:
  - Invert the microparticle bottle 30 times.
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott Representative.**
- Order tests.
- Load the ARCHITECT CA 15-3 Reagent Kit on the ARCHITECT *i* System. Verify that all necessary assay reagents are present. Ensure that septums are present on all reagent bottles.
- The minimum sample volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
  - Patient specimen priority: 70 µL for the first CA 15-3 test plus 20 µL for each additional test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first CA 15-3 test plus 20 µL for each additional CA 15-3 test from the same sample cup.
  - > 3 hours on board: additional sample volume is required. Refer to the Architect System Operations Manual, Section 5 for information on sample evaporation and volumes.
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- ARCHITECT CA 15-3 Calibrators and Controls should be mixed by gentle inversion prior to use.
  - To obtain the recommended 250 µL volume requirements for the ARCHITECT CA 15-3 Calibrators, hold the bottles **vertically** and dispense 7 drops of each calibrator into each respective sample cup.
  - To obtain the recommended 150 µL volume requirements for the ARCHITECT CA 15-3 Controls, hold the bottles **vertically** and dispense 4 drops of each control into each respective sample cup.
- Load samples
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN. The ARCHITECT *i* System performs the following function:
  - Moves the sample to the aspiration point
  - Loads a reaction vessel (RV) into the process path
  - Aspirates and transfers sample into the RV
  - Advances the RV one position
  - Aspirates wash buffer and microparticles and transfers them into the RV (for specimen or control) or aspirates microparticles and transfers them into the RV (for a prediluted calibrator).
  - Mixes, incubates and washes the reaction mixture
  - Adds conjugate to the RV
  - Mixes, incubates and washes the reaction mixture
  - Adds Pre-Trigger and Trigger Solutions
  - Measures chemiluminescent emission to determine the quantity of DF3 defined antigen in the sample
  - Aspirates contents of RV to liquid waste and unloads RV to solid waste
  - Calculates the result
- For information on ordering patient specimens and controls, and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- It is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory procedures require more frequent maintenance, follow those procedures.

### Specimen Dilution Procedures

Specimens with a CA 15-3 assay value exceeding 800 U/mL are flagged with the code “ >800.0” and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

- If using the Automated Dilution Protocol, the system performs a 1:5 dilution of the specimen and automatically calculates the concentration of the sample before dilution and reports the result.
- Manual dilutions should be performed as follows:
  - The suggested dilution for the ARCHITECT CA 15-3 assay is 1:5.
  - For a 1:5 dilution, add 100 µL of the patient specimen to 400 µL of ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
  - The operator must enter the dilution factor in the Patient or Control order screen. All assays selected for that order will be diluted. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The dilution should be performed so that the diluted result reads greater than 30 U/mL.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

### Calibration

- To perform an ARCHITECT CA 15-3 calibration, test calibrators A, B, C, D, E, and F in duplicate. A single sample of all levels of CA 15-3 controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- Calibration range: 0 - 800 U/mL.
- Once an ARCHITECT CA 15-3 calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used
  - Controls are out of range
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

### QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT CA 15-3 assay is that a single sample of each control be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. Ensure that assay control values are within the concentration ranges specified in the package insert.

### Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT CA 15-3 assay belongs to method group 1.

### RESULTS

#### Calculation

- The ARCHITECT CA 15-3 assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

#### Flags

- Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

#### Measurement Range (Reportable Range)

- The measurement range for the ARCHITECT CA 15-3 assay is 0.5 U/mL to 800 U/mL.

### LIMITATIONS OF THE PROCEDURE

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- If the ARCHITECT CA 15-3 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.<sup>25,26</sup> Additional clinical or diagnostic information may be required to determine patient status.

- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.<sup>27</sup> Additional information may be required for diagnosis.
- Patients with confirmed breast carcinoma may have CA 15-3 assay values in the same range as healthy individuals. Elevations in circulating DF3 defined antigen may be observed in patients with nonmalignant disease. For these reasons, a CA 15-3 assay value, regardless of level, should not be interpreted as absolute evidence for the presence or absence of malignant disease. The CA 15-3 assay value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures. **The ARCHITECT CA 15-3 assay should not be used as a cancer screening test.**
- The ARCHITECT CA 15-3 Calibrators are supplied prediluted. A specialized protocol dilutes all controls and specimens by the same final dilution factor as the prediluted calibrators.
- Representative performance data are given in the **EXPECTED VALUES and SPECIFIC PERFORMANCE CHARACTERISTICS** sections. Results obtained in individual laboratories may vary.

#### EXPECTED VALUES

The distribution of CA 15-3 assay values determined in 396 specimens is shown in the table below:

Distribution of ARCHITECT CA 15-3 Assay Values					
	Number of Subjects	Percent (%)			
		0-31.3 U/mL	31.4-60 U/mL	60.1-120 U/mL	>120 U/mL
<b>APPARENTLY HEALTHY</b>					
Females (Premenopausal)	99	99.0	1.0	0.0	0.0
Females (Postmenopausal)	100	99.0	0.0	1.0	0.0
Males	197	98.5	1.5	0.0	0.0
<b>Total</b>	<b>396</b>	<b>98.7</b>	<b>1.0</b>	<b>0.3</b>	<b>0.0</b>

In this study, 99.0% of the healthy female subjects had CA 15-3 assay values at or below 31.3 U/mL (mean = 13.0, SD = 7.0).

It is recommended that each laboratory establish its own reference value for the population of interest.

#### Monitoring of Disease Status in Patients Diagnosed with Breast Cancer

Changes observed in serial CA 15-3 assay values, when monitoring breast cancer patients, should be evaluated in conjunction with other clinical methods used for monitoring breast cancer.

The effectiveness of ARCHITECT CA 15-3 assay as an aid in the monitoring of disease status in patients diagnosed with breast cancer was determined by assessing changes in CA 15-3 levels in serial serum samples with changes in disease status. A study involving samples from 74 patients with a total of 377 observations was performed. The average number of observations per patient was 5.1. A significant change in a CA 15-3 concentration was defined as an increase in the value that was at least a 9.575% increase in assay value [i.e., 2.5 times greater than the assay's total %CV (3.83%)]. Seventy-six percent (76% or 50/66) of the patients with significant serial sample increases correlated with disease progression while sixty-five percent (65% or 153/237) of serial samples showing no significant change in the CA 15-3 value, correlated with no progression. The total concordance in this study was sixty-seven percent (67% or 203/303). The following table presents the data in a 2 x 2 classification scheme.

Change in Disease State per Sequential Pair			
Change in CA 15-3			
Concentration	Progression	No Progression	Total
≥ 9.575%	50	84	134
< 9.575%	16	153	169
<b>Total</b>	<b>66</b>	<b>237</b>	<b>303</b>

The following table demonstrated the per patient distribution. Ninety-seven percent (97% or 36/37) of the significantly increased serial samples correlated with disease progression while twenty-seven percent (27% or 10/37) of serial samples, showing no significant change in the CA 15-3 concentration, correlated with no progression. The total concordance in this study was sixty-two percent (62% or 46/74).

Change in Disease State per Patient			
Change in CA 15-3			
Concentration	Progression	No Progression	Total
≥ 9.575%	36	27	63
< 9.575%	1	10	11
<b>Total</b>	<b>37</b>	<b>37</b>	<b>74</b>

#### SPECIFIC PERFORMANCE CHARACTERISTICS

##### Precision

The ARCHITECT CA 15-3 assay precision is ≤ 8% total CV. A study was performed as described per the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-A.<sup>28</sup> Five defibrinated plasma-based panels were assayed, using two lots of reagents, in replicates of two at two separate times per day for 20 days on two separate instruments. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized below.\*

Panel Member	Reagent Lot	Instrument	n	Mean Conc. (U/mL)	Within Run		Total	
					SD	%CV	SD	%CV
1	1	1	80	28.6	0.7	2.4	0.9	3.1
	2	2	80	27.0	0.6	2.2	0.7	2.6
2	1	1	80	121.2	3.6	3.0	4.5	3.7
	2	2	80	112.6	1.9	1.7	2.4	2.2
3	1	1	80	251.6	7.9	3.1	9.5	3.8
	2	2	80	229.9	4.2	1.8	6.5	2.8
4	1	1	80	494.7	18.2	3.7	22.3	4.5
	2	2	80	477.8	12.7	2.7	17.3	3.6
5	1	1	80	683.1	32.2	4.7	34.6	5.1
	2	2	80	666.2	20.1	3.0	29.5	4.4

\* Representative data; results in individual laboratories may vary from these data.

##### Recovery

The ARCHITECT CA 15-3 assay mean recovery is 100 ± 15%. A study was performed based on guidance from Tietz Textbook of Clinical Chemistry<sup>29</sup> for the ARCHITECT CA 15-3 assay. Known concentrations of DF3 defined antigen were added to normal human serum samples. The concentration of CA 15-3 was determined using the ARCHITECT CA 15-3 assay, and the resulting percent recovery was calculated. Representative data from this study are summarized in the table below.\*

Sample	Endogenous Assay Value (U/mL)	DF3 Defined Antigen Added (U/mL)	Observed CA 15-3 Assay Value (U/mL)	Percent Recovery <sup>a</sup>
1	31.3	45	73.0	96
		365	381.6	96
2	30.9	45	63.7	84
		365	356.8	90
3	34.3	45	69.8	88
		365	386.0	97

Average recovery across two separate spiked concentrations shown above = 91.8%

$$^a \text{ \% Recovery} = \frac{\text{Observed CA 15-3 Conc. (U/mL)}}{\text{Endogenous CA 15-3 Conc. (U/mL) + CA 15-3 Added (U/mL)}} \times 100$$

\* Representative data; results in individual laboratories may vary from these data.

##### Dilution Linearity

The ARCHITECT CA 15-3 assay mean dilution linearity is 100 ± 15%. A study was performed for the ARCHITECT CA 15-3 assay modeled after the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP6-P2<sup>30</sup>. Samples with known elevated CA 15-3 concentrations were diluted with Multi-Assay Manual Diluent. The CA 15-3 concentration was determined for each dilution and the percent (%) recovery was calculated. Representative data from this study are summarized in the following table.\*

Sample	Final Dilution Factor	Expected Value (U/mL)	Value Obtained (U/mL)	% Recovery <sup>a</sup>
1	Undiluted	680.4	680.4	-
	1:1.4	687.3	490.9	101
	1:2	645.4	322.7	95
	1:3.3	610.2	184.9	90
	1:5	634.0	126.8	93
	1:10	603.0	60.3	89
2	Undiluted	754.8	754.8	-
	1:1.4	708.1	505.8	94
	1:2	648.0	324.0	86
	1:3.3	655.1	198.5	87
	1:5	646.5	129.3	86
	1:10	607.0	60.7	80
3	Undiluted	705.0	705.0	-
	1:1.4	702.5	501.8	100
	1:2	705.8	352.9	100
	1:3.3	657.7	199.3	93
	1:5	702.0	140.4	100
	1:10	668.0	66.8	95

Average recovery across the three diluted samples above = 92%

$$^a \text{ \% Recovery} = \frac{\text{Values Obtained} \times \text{Dilution Factor}}{\text{Undiluted Concentration}} \times 100$$

\* Representative data; results in individual laboratories may vary from these data.

#### Analytical Sensitivity

The sensitivity of the ARCHITECT CA 15-3 assay is  $\leq 0.5$  U/mL (n=24 runs, in replicates of 10). Analytical sensitivity corresponds to the upper limit of the 95% confidence interval and represents the lowest concentration of DF3 defined antigen that can be distinguished from zero.

#### Analytical Specificity

The ARCHITECT CA 15-3 mean assay specificity is  $\leq 12\%$ . Recovery studies were performed to compare sera containing the following compounds at the indicated concentrations with control sera.\*

#### INTERFERING SUBSTANCE

Test Compound	Test Concentration
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Total Protein	12 g/dL
Triglycerides	3 g/dL

#### CHEMOTHERAPEUTIC AGENTS

Test Compound	Test Concentration
Beta Estradiol	6.7 µg/mL
Cisplatin	66.7 µg/mL
Cyclophosphamide	330 µg/mL
Doxorubicin	6.6 µg/mL
5-fluorouracil	280 µg/mL
Megestrol Acetate	39.6 µg/mL
Methotrexate	13.2 µg/mL
Mitomycin C	17.2 µg/mL
Paclitaxel	3.5 ng/mL
Tamoxifen	5.0 µg/mL
Testosterone	33.0 µg/mL
Vinblastine sulfate	1.3 µg/mL

#### POTENTIALLY INTERFERING CLINICAL CONDITIONS

The ARCHITECT CA 15-3 assay was evaluated using specimens with HAMA and Rheumatoid Factor (RF) to further assess the assay specificity. Five specimens positive for HAMA and five specimens positive for RF were evaluated for % recovery with DF3 defined antigen spiked into each specimen at 35 and 250 U/mL; mean % recovery results are summarized in the following table.\*

Clinical Condition	Number of Specimens	Mean % Recovery
HAMA	10	108
RF	10	103

\* Representative data; results in individual laboratories may vary from these data.

#### High Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the ARCHITECT CA 15-3 assay, no high dose hook effect was observed when samples containing up to approximately 22,000 U/mL of DF3 defined antigen were assayed.

#### Method Comparison

The ARCHITECT CA 15-3 assay method comparison correlation coefficient is  $\geq 0.90$  and the slope is  $1.0 \pm 0.15$ . The ARCHITECT CA 15-3 assay was compared to the Abbott AxSYM CA 15-3 assay. The results of the specimen testing are shown in the following table.\*

ARCHITECT CA 15-3 vs. Abbott AxSYM CA 15-3				
Regression Method	Number of Specimens	Correlation Coefficient	Intercept (99% CI)	Slope (99% CI)
Passing-Bablok †	402	0.980	-0.3 (-0.9 to 0.0)	0.94 (0.92 to 0.97)

Sample Range (ARCHITECT): 0.0-1326.5 U/mL

Sample Range (AxSYM): 4.9-1621.9 U/mL

† A linear regression method with no special assumptions regarding the distribution of the samples and the measurement errors.<sup>31</sup>

\* Representative data; results in individual laboratories may vary from these data.

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